Journal of Peptide Science
J. Peptide Sci. **11**: 812–817 (2005)
Published online 15 August 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.685

JOURNAL OF PEPTIDE SCIENCE

The first synthesis of chiral PNA monomer-cyclen conjugates

LI-JIAN MA,[a] GUO-LIN ZHANG,[b] SHAN-YONG CHEN,[a] BO WU,[a] JING-SONG YOU,[a] CHUAN-QIN XIA[a] and XIAO-QI YU[a]*

[a] Department of Chemistry, Key Laboratory of Green Chemistry and Technology (Ministry of Education), Sichuan University, Chengdu 610064, P. R. China
[b] Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu 610041, P. R. China

Received 18 November 2004; Accepted 1 March 2005

Abstract: A synthetic route to novel chiral PNA monomer-cyclen conjugates was described for the first time, the targeted products were obtained in high yields under mild reaction conditions. The preliminary results demonstrated that the uracil-PNA monomer-cyclen conjugates can rapidly bind Zn^{2+} in aqueous solution, and the structure of the Zn(II) complex was confirmed facilely by HRMS spectra, ^{1}H NMR spectra and elemental analysis. Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptide nucleic acids (PNA); 1,4,7,10-tetraazacyclododecane (cyclen); synthesis; conjugates

INTRODUCTION

Peptide nucleic acid (PNA), reported in 1991 by Nielsen [1,2], is an oligonucleotide analogue in which the sugar-phosphate backbone is replaced by a polyamide chain linked to the nucleobase (Figure 1). PNA offers important advantages for the recognition of nucleic acids including high-affinity binding, resistance to degradation by nucleases or proteases, and a low affinity for proteins [3,4]. Structural characterizations [5,6] show that when PNA is complexed with a natural nucleic acid the internal amide bonds of the PNA units are uniformly aligned so that the carbonyl oxygens of the tertiary amide bond point to the carboxy terminus in an antiparallel Watson–Crick duplex. Many derivatives and analogues of PNA composed of *N*-(2-aminoethyl)glycine units were also designed and prepared to improve their physico-chemical and biological properties [7,8]. Recently the introduction of chirality into the backbone has attracted much attention [9–11]. Howarth and Wakelin reported that α-PNAs, in which the thymine, cytosine, adenine and guanine base-containing amino acid moieties are derived from homoserine, are to modify the backbone electrostatic charge, hydrophobicity and ridigity by replacing glycine residues with appropriate amino acids [9]. This study describes in detail the synthesis of a uracil base-containing amino acid from L-cysteine and their subsequent condensation with natural L-amino acids to form a novel chiral uracil-PNA monomer. These monomers can be conveniently conjugated with cyclen.

1,4,7,10-Tetraazacyclododecane (cyclen) has become an important building block for the synthesis of diagnostic and therapeutic pharmaceutical agents within the past decade [12,13]. One of the fastest growing medicinal uses of cyclen is in the development of MRI contrast agents [13,14]. More recently, advances in targeted cancer agents such as antibodies and peptides have revived interest in cyclen-based bifunctional chelating agents for therapy [15]. The mononuclear and dinuclear Zn(II) complexes of cyclens are also found to be efficient DNA cleavage agents [16–18].

In an effort to increase the specificity and to direct the cleavage to within the minor groove of DNA, the recognition and cleavage functions to DNA or RNA in the same small molecule are desired greatly. This study presents a type of novel chiral PNA monomer-cyclen conjugates which may possibly combine the properties of PNA and cyclen. The uracil as a recognition element was linked to dipeptide to form chiral PNA monomers and cyclen as a functional moiety was introduced to construct the conjugates. To the best of our knowledge, this is the first example of chiral PNA monomer-cyclen conjugates.

MATERIALS AND METHODS

ESI-MS and HRMS spectra data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer respectively. The ^{1}H NMR spectra were measured on a Varian INOVA-400 spectrometer and chemical shifts in ppm are reported relative to internal Me_4Si ($CDCl_3$, DMSO-d_6) or 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (D_2O). Elemental analyses were performed using a Carlo-Elba 1106 elemental analytical instrument. Polarimetric measurements were taken on a Perkin-Elmer-341 automatic polarimeter. Melting points were determined using a micro-melting point apparatus and are

* Correspondence to: X-Q Yu, Department of Chemistry, Key Laboratory of Green Chemistry and Technology (Ministry of Education), Sichuan University, Chengdu 610064, P. R. China;
e-mail: schemorg@mail.sc.cninfo.net

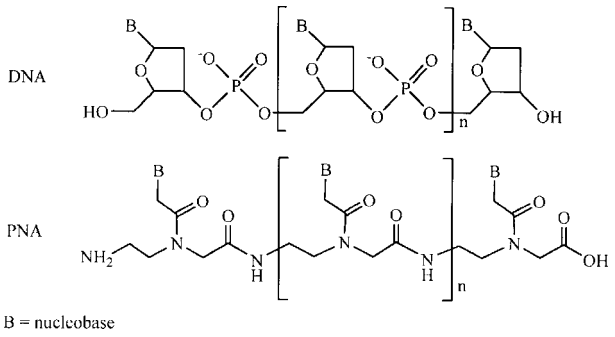


Figure 1 Comparison of structure of DNA and PNA.

uncorrected. 5-Hydroxymethyluracil [19] and 1,4,7-tris (*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecance (3Boc-cyclen) [20] were prepared according to the literature. All other chemicals and reagents were obtained commercially and were used without further purification.

S-Thyminyl-L-cysteine hydrochloride 3

A solution of L-cysteine hydrochloride (0.852 g, 6.00 mmol) and 5-hydroxymethyluracil (0.942 g, 6.00 mmol) in 2 N HCl (30 ml) was stirred at 50 °C for 48 h. After cooling, filtering and evaporating, the S-thyminyl-L-cysteine hydrochloride was obtained as a white powder (1.59 g, 94%). Mp 238°–240 °C; $[\alpha]^{20}_D = +9.8$ ($c = 1.0$, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 7.64 (s, 1 H, uracil-6-C<u>H</u>), 4.36–4.33 (m, 1 H, $NH_2C\underline{H}$), 3.59 (s, 2 H, S-C<u>H_2</u>-uracil), 3.28–3.23 (m, 1 H, NCHC<u>H_2</u>S), 3.15–3.09 (m, 1 H, NCHC<u>H_2</u>S); ESI-MS: $m/z =$ 244.0 [M-1-HCl]$^-$; HRMS (ESI) calcd for $C_8H_{11}N_3O_4SNa$ [M + Na-HCl]$^+$: $m/z = 268.0362$. Found: 268.0355.

N-*tert*-Butyloxycarbonyl-S-thyminyl-L-cysteine 4

To an aqueous solution of sodium hydrogen carbonate (1.68 g, 20.0 mmol); S-thyminyl-L-cysteine hydrochloride (1.12 g, 4.00 mmol) in 1,4-dioxane (60 ml) was added. The solution was then cooled to 0 °C with an ice bath. Di-*t*-butyl carbonate (2.62 g, 12.0 mmol) dissolved in 1,4-dioxane (20 ml) was added dropwise. After being kept at this temperature for another 20 h, the solution was allowed to warm to room temperature. The reaction mixture was concentrated under reduced pressure and the pH was adjusted to 3–4 with 1 N $KHSO_4$. After cooling and filtering, compound **4** was obtained as a white powder (1.324 g, 96%). Mp 118°–119 °C; $[\alpha]^{20}_D = +11.1$ ($c = 1.0$, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.82 (s, 1 H, COO<u>H</u>), 11.14 (s, 1 H, uracil-1-N<u>H</u>), 10.85 (s, 1 H, uracil-3-N<u>H</u>), 7.41 (s, 1 H, uracil-6-C<u>H</u>), 6.92 (s, 1 H, OCONH), 4.04–3.99 (m, 1 H, Boc-NHC<u>H</u>), 2.89–2.79 (m, 2 H, S-C<u>H_2</u>-uracil), 2.73–2.65 (m, 2 H, NCHC<u>H_2</u>S), 1.38 [s, 9 H, C(C<u>H_3</u>)$_3$]; ESI-MS: $m/z =$ 344.4 [M-1]$^-$; HRMS (ESI) calcd for $C_{13}H_{19}N_3O_6SNa$ [M + Na]$^+$: $m/z = 368.0887$. Found: 368.0889.

General Procedure for the Preparation of 5

To a solution of *N*-*tert*-butyloxycarbonyl-S-thyminyl-L-cysteine (1.03 g, 3.00 mmol) in anhydrous tetrahydrofuran (THF, 40 ml), *N*-methyl morphine (NMM) (0.390 ml, 3.30 mmol) and *i*-C_4H_9OCOCl (0.402 ml, 3.00 mmol) were added sequentially at about −15 °C. After 10 min a solution of L-amino acid methyl ester (3.30 mmol) and NMM (0.390 ml, 3.30 mmol) in anhydrous THF (60 ml) was poured into the reaction mixture. The mixture was stirred for another 0.5 h at −15 °C and left overnight at room temperature. After the solvent was evaporated under reduced pressure, the solid residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$, saturated brine and 2 N citric acid and the organic layer was dried with anhydrous Na_2SO_4. After the solvent was removed, the residue was purified by recrystallization from acetone and ethyl ether. Compound **5** was obtained as a white powder.

5a: 1.136 g, 91%. Mp 199°–201 °C; $[\alpha]^{20}_D = +8.5$ ($c = 1.0$, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1 H, uracil-1-N<u>H</u>), 10.82 (d, 1 H, $J = 4.4$ Hz, uracil-3-N<u>H</u>), 8.37 (t, 1 H, $J = 6.0$ Hz, CON<u>H</u>), 7.40 (d, 1 H, $J =$ 6.0 Hz, uracil-6-C<u>H</u>), 6.99 (d, 1 H, $J = 8.8$ Hz, OCON<u>H</u>), 4.18–4.12 (m, 1 H, Boc-NHC<u>H</u>), 3.86–3.83 (m, 2 H, C<u>H_2</u>COOC<u>H_3</u>), 3.62 (s, 3 H, OC<u>H_3</u>), 2.77–2.56 (m, 2 H, S-C<u>H_2</u>-uracil), 2.55–2.50 (m, 2 H, NCHC<u>H_2</u>S), 1.38 [s, 9 H, C(C<u>H_3</u>)$_3$]; ESI-MS: $m/z = 415.1$ [M-1]$^-$; HRMS (ESI) calcd for $C_{16}H_{24}N_4O_7SNa$ [M + Na]$^+$: $m/z = 439.1258$. Found: 439.1243.

5b: 1.317 g, 93%. Mp 205°–206 °C; $[\alpha]^{20}_D = +7.0$ ($c = 1.0$, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.16 (s, 1 H, uracil-1-N<u>H</u>), 10.84 (s, 1 H, uracil-3-N<u>H</u>), 8.24 (d, 1 H, $J = 7.6$ Hz, CON<u>H</u>), 7.41 (s, 1 H, uracil-6-C<u>H</u>), 6.98 (d, 1 H, $J = 8.4$ Hz, OCON<u>H</u>), 4.31–4.26 (m, 1 H, C<u>H</u>COOCH$_3$), 4.15–4.10 (m, 1 H, Boc-NHC<u>H</u>), 3.60 (s, 3 H, OC<u>H_3</u>), 2.74–2.56 (m, 2 H, S-C<u>H_2</u>-uracil), 2.55–2.50 (m, 2 H, NCHC<u>H_2</u>S), 1.64–1.54 (m, 2 H, CHC<u>H_2</u>CH), 1.52–1.45 [m, 1 H, C<u>H</u>(CH$_3$)$_2$], 1.38 [s, 9 H, C(C<u>H_3</u>)$_3$], 0.89 (d, 3 H, $J = 6.4$ Hz, CHC<u>H_3</u>), 0.83 (d, 3 H, $J =$ 6.4 Hz, CHC<u>H_3</u>); ESI-MS: $m/z = 471.5$ [M-1]$^-$; HRMS (ESI) calcd for $C_{20}H_{32}N_4O_7SNa$ [M + Na]$^+$: $m/z = 495.1884$. Found: 495.1876.

5c: 1.378 g, 88%. Mp 128°–130 °C; $[\alpha]^{20}_D = +10.2$ ($c = 1.0$, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.17 (s, 1 H, uracil-1-N<u>H</u>), 10.85 (d, 1 H, $J = 4.8$ Hz, uracil-3-N<u>H</u>), 9.24 (s, 1 H, Ph-O<u>H</u>), 8.21 (d, 1 H, $J = 7.6$ Hz, CON<u>H</u>), 7.41 (d, 1 H, $J = 6.0$ Hz, uracil-6-C<u>H</u>), 6.98 (d, 2 H, $J = 8.4$ Hz, Ph-<u>H</u>), 6.94 (d, 1 H, $J = 8.8$ Hz, OCON<u>H</u>), 6.64 (d, 2 H, $J = 8.4$ Hz, Ph-<u>H</u>), 4.40–4.35 (m, 1 H, C<u>H</u>COOCH$_3$), 4.17–4.11 (m, 1 H, Boc-NHC<u>H</u>), 3.56 (s, 3 H, OC<u>H_3</u>), 2.92–2.83 (m, 2 H, Ph-C<u>H_2</u>), 2.71–2.54 (m, 2 H, S-C<u>H_2</u>-uracil), 2.51–2.49 (m, 2 H, NCHC<u>H_2</u>S), 1.38 [s, 9 H, C(C<u>H_3</u>)$_3$]; ESI-MS: $m/z = 521.1$ [M-1]$^-$; HRMS (ESI) calcd for $C_{23}H_{30}N_4O_8SNa$ [M + Na]$^+$: $m/z = 545.1677$. Found: 545.1689.

General Procedure for the Preparation of 6

Sodium hydroxide 2 N (5 ml, 10 mmol) was added slowly dropwise to a suspension of compound **5** (2.00 mmol) in methanol (20 ml) at 0 °C. The mixture was stirred for 2 h at room temperature and the pH was adjusted to 7 with 1 N HCl. After removing most of the methanol, the pH was adjusted to about 2. The mixture was then extracted with ethyl acetate (3 × 30 ml) and the organic phase was dried with anhydrous Na_2SO_4. After the solvent was evaporated under reduced pressure, compound **6** was obtained as a white solid.

6a: 0.764 g, 95%. Mp 195°–197 °C; $[\alpha]^{20}_D = +13.2$ ($c = 1.0$, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ 12.60 (s, 1 H, COOH), 11.13 (s, 1 H, uracil-1-NH), 10.83 (d, 1 H, J = 4.0 Hz, uracil-3-NH), 8.24 (t, 1 H, $J = 5.6$ Hz, CONH), 7.42 (d, 1 H, $J = 5.6$ Hz, uracil-6-CH), 6.95 (d, 1 H, $J = 8.8$ Hz, OCONH), 4.19–4.14 (m, 1 H, Boc-NHCH), 3.80–3.72 (m, 2 H, CH_2COOH), 2.79–2.54 (m, 2 H, S-CH_2-uracil), 2.51–2.49 (m, 2 H, NCHCH_2S), 1.38 [s, 9 H, C(CH_3)$_3$]; ESI-MS: $m/z = 401.0$ [M-1]$^-$; HRMS (ESI) calcd for $C_{15}H_{22}N_4O_7SNa$ [M + Na]$^+$: $m/z = 425.1101$. Found: 425.1099.

6b: 0.898 g, 98%. Mp 201°–203 °C; $[\alpha]^{20}_D = +9.3$($c = 1.0$, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ12.52 (s, 1 H, COOH), 11.13 (s, 1 H, uracil-1-NH), 10.79 (d, 1 H, $J = 4.4$ Hz, uracil-3-NH), 8.03 (d, 1 H, $J = 8.0$ Hz, CONH), 7.41 (d, 1 H, $J = 5.6$ Hz, uracil-6-CH), 6.95 (d, 1 H, $J = 8.4$ Hz, OCONH), 4.27–4.20 (m, 1 H, CHCOOH), 4.15–4.10 (m, 1 H, Boc-NHCH), 2.76–2.54 (m, 2 H, S-CH_2-uracil), 2.52–2.49 (m, 2 H, NCHCH_2S), 1.65–1.53 (m, 2 H, CHCH_2CH), 1.52–1.44 [m, 1 H, CH(CH_3)$_2$], 1.38 [s, 9 H, C(CH_3)$_3$], 0.89–0.82 [m, 6 H, CH(CH_3)$_2$]; ESI-MS: $m/z = 457.1$ [M-1]$^-$; HRMS (ESI) calcd for $C_{19}H_{30}N_4O_7SNa$ [M + Na]$^+$: $m/z = 481.1728$. Found: 481.1725.

6c: 0.996 g, 98%. Mp 101°–102 °C; $[\alpha]^{20}_D = +10.7$ ($c = 1.0$, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6): δ12.72 (s, 1 H, COOH), 11.15 (s, 1 H, uracil-1-NH), 10.81 (d, 1 H, $J = 4.8$ Hz, uracil-3-NH), 9.20 (s, 1 H, Ph-OH), 7.94 (d, 1 H, $J = 7.6$ Hz, CONH), 7.40 (d, 1 H, $J = 5.6$ Hz, uracil-6-CH), 6.99 (d, 2 H, $J = 8.4$ Hz, Ph-H), 6.94 (d, 1 H, $J = 8.8$ Hz, OCONH), 6.62 (d, 2 H, $J = 8.4$ Hz, Ph-H), 4.36–4.32 (m, 1 H, CHCOOH), 4.15–4.10 (m, 1 H, Boc-NHCH), 2.94–2.82 (m, 2 H, Ph-CH_2), 2.78–2.54 (m, 2 H, S-CH_2-uracil), 2.52–2.50 (m, 2 H, NCHCH_2S), 1.38 [s, 9 H, C(CH_3)$_3$]; ESI-MS: $m/z = 507.2$[M-1]$^-$; HRMS (ESI) calcd for $C_{22}H_{28}N_4O_8SNa$ [M + Na]$^+$: m/z = 531.1521. Found: 531.1543.

General Procedure for the Preparation of 7

To a solution of compound **6** (1.00 mmol) in anhydrous THF (20 ml), *N*-methyl morphine (NMM) (0.130 ml, 1.10 mmol) and *i*-C_4H_9OCOCl (0.134 ml, 1.00 mmol) were added at about −15 °C. After 10 min a solution of 3Boc-cyclen (0.472 g, 1.00 mmol) in anhydrous THF (30 ml) was poured into the reaction mixture. The mixture was stirred for another 0.5 h at −15 °C and left overnight at room temperature. The mixture was then evaporated under reduced pressure to remove the solvent. The solid residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$, saturated brine and 2 N citric acid and the organic layer was dried with anhydrous Na_2SO_4. After the solvent was evaporated, the residue was purified by column chromatography on silica gel (eluent: ethyl acetate/petroleum ether/acetone = 1 : 1 : 1, v/v/v) to afford compounds **7** as a white solid.

7a: 0.565 g, 66%. Mp 134°–136 °C; $[\alpha]^{20}_D = +4.7$ ($c = 1.0$, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ10.40 (s, 1 H, uracil-1-NH), 10.24 (s, 1 H, uracil-3-NH), 7.59 (s, 1 H, CONH), 7.54 (s, 1 H, uracil-6-CH), 5.72 (d, 1 H, $J = 7.6$ Hz, OCONH), 4.49–4.47 (m, 1 H, Boc-NHCH), 4.13–4.08 (m, 2 H, CH_2CON), 3.53–3.40 (m, 16 H, cyclen-CH_2), 2.92–2.80 (m, 2 H, S-CH_2-uracil), 2.52–2.50 (m, 2 H, NCHCH_2S), 1.47–1.43 (m, 36 H, Boc-H); ESI-MS: $m/z = 855.5$ [M-1]$^-$; HRMS (ESI) calcd for $C_{38}H_{64}N_8O_{12}SNa$ [M + Na]$^+$: $m/z = 879.4257$. Found: 879.4289.

7b: 0.584 g, 64%. Mp 137°–139 °C; $[\alpha]^{20}_D = +3.2$ ($c = 1.0$, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ 10.46 (s, 1 H, uracil-1-NH), 10.20 (s, 1 H, uracil-3-NH), 7.66 (s, 1 H, CONH), 7.40 (s, 1 H, uracil-6-CH), 5.77 (d, 1 H, J = 7.6 Hz, OCONH), 4.81–4.70 (m, 1 H, CHCON), 4.52–4.47 (m, 1 H, Boc-NHCH), 3.72–3.40 (m, 16 H, cyclen-CH_2), 2.75–2.68 (m, 2 H, S-CH_2-uracil), 2.63–2.57 (m, 2 H, NCHCH_2S), 1.67–1.64 (m, 2 H, CHCH_2CH), 1.62–1.51 [m, 1 H, CH(CH_3)$_2$], 1.46–1.42 (m, 36 H, Boc-H), 0.99–0.83 [m, 6 H, CH(CH_3)$_2$]; ESI-MS: $m/z = 911.6$ [M-1]$^-$; HRMS (ESI) calcd for $C_{42}H_{72}N_8O_{12}SNa$ [M + Na]$^+$: m/z = 935.4883. Found: 935.4896.

7c: 0.577 g, 60%. Mp 130°–131 °C; $[\alpha]^{20}_D = +4.3$ ($c = 1.0$, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ 10.34 (s, 1 H, uracil-1-NH), 10.22 (s, 1 H, uracil-3-NH), 7.80 (d, 1 H, $J = 8.0$ Hz, CONH), 7.70 (d, 1 H, $J = 8.0$ Hz, uracil-6-CH), 7.37–7.30 (m, 2 H, Ph-H), 7.02–6.96 (m, 1 H, OCONH), 6.76–6.69 (m, 2 H, Ph-H), 5.02–5.00 (m, 1 H, CHCON), 4.42–4.39 (m, 1 H, Boc-NHCH), 3.70–3.17 (m, 16 H, cyclen-CH_2), 2.92–2.85 (m, 2 H, Ph-CH_2), 2.76–2.72 (m, 2 H, S-CH_2-uracil), 2.64–2.61 (m, 2 H, NCHCH_2S), 1.45–1.42 (m, 36 H, Boc-H); ESI-MS: $m/z = 961.4$ [M-1]$^-$; HRMS (ESI) calcd for $C_{45}H_{70}N_8O_{13}SNa$ [M + Na]$^+$: m/z = 985.4675. Found: 985.4638.

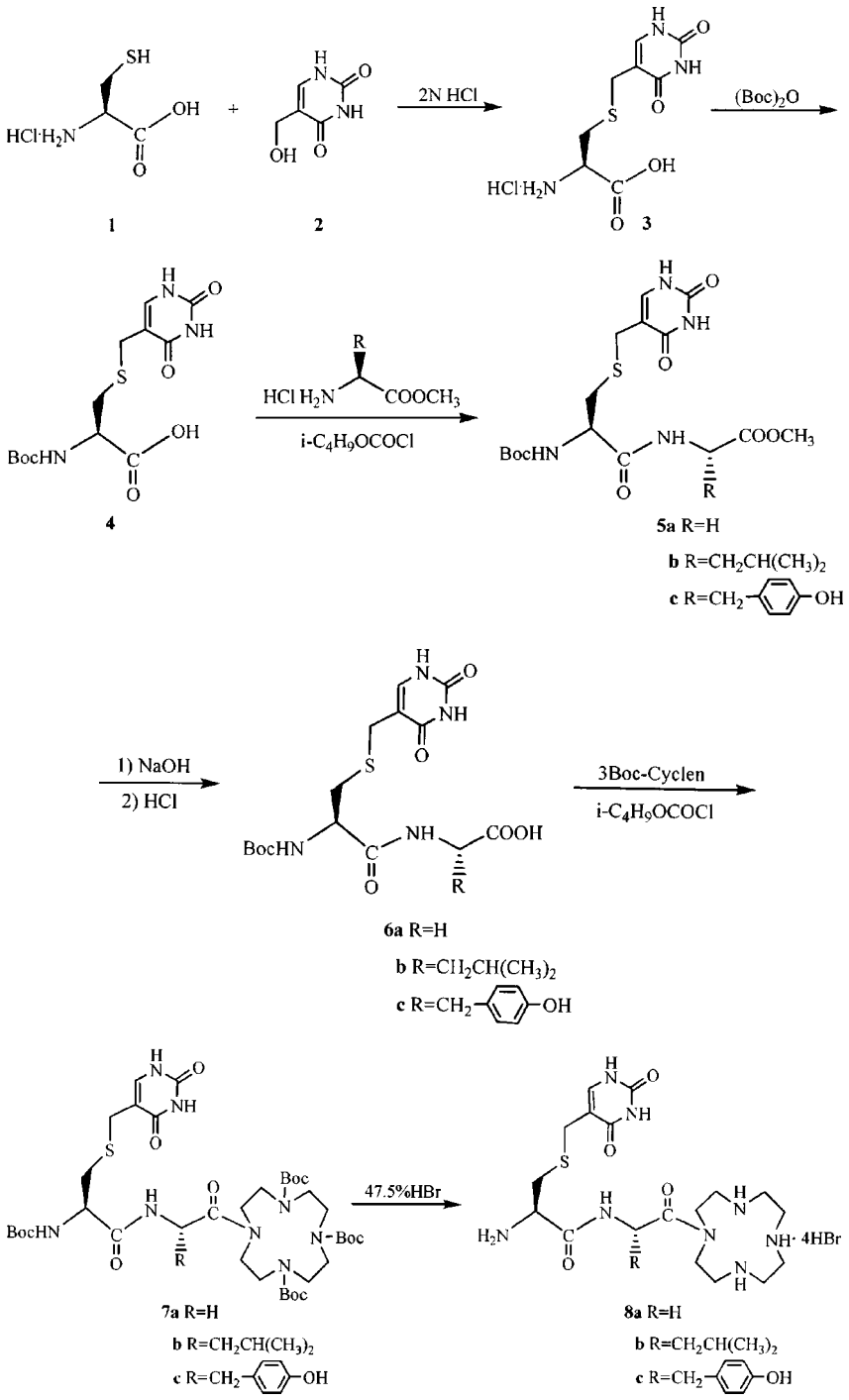
General Procedure for the Preparation of 8

To a solution of compound 7 (0.500 mmol) in ethanol (10 ml), 47.5% aqueous HBr (5 ml) was added dropwise. After being stirred at room temperature overnight, the reaction mixture was concentrated under reduced pressure to give the crude product, which was crystallized from ethanol/24% aqueous HBr to afford compound **8** as a white powder.

8a: 0.303 g, 78%. Mp 188°–190 °C; $[\alpha]^{20}_D = +1.4$ ($c = 1.0$, H_2O); ^1H NMR (400 MHz, D_2O): δ 7.67 (s, 1 H, uracil-6-CH), 4.46–4.43 (m, 1 H, NH_2CH), 4.30 (s, 2 H, CH_2CON), 3.82–3.29 (m, 16 H, cyclen-CH_2), 3.25–3.19 (m, 2 H, S-CH_2-uracil), 3.11–3.05 (m, 2 H, NCHCH_2S); ESI-MS: $m/z = 455.2$ [M-1-4HBr]$^-$; Anal. Calcd for $C_{18}H_{36}N_8O_4Br_4S$: C, 27.71; H, 4.65; N, 14.36. Found: C, 27.70; H, 4.86; N, 14.54.

8b: 0.329 g, 79%. Mp 243°–245 °C; $[\alpha]^{20}_D = +2.2$ ($c = 1.0$, H_2O); ^1H NMR (400 MHz, D_2O): δ 7.71–7.68 (m, 1 H, uracil-6-CH), 4.68–4.63 (m, 1 H, CHCON), 4.50–4.42 (m, 1 H, NH_2CH), 3.82–3.27 (m, 16 H, cyclen-CH_2), 3.26–3.17 (m, 2 H, S-CH_2-uracil), 3.15–3.06 (m, 2 H, NCHCH_2S) 1.84–1.73 (m, 2 H, CHCH_2CH), 1.65–1.59 [m, 1 H, CH(CH_3)$_2$], 1.05–1.00 [m, 6 H, CH(CH_3)$_2$]; ESI-MS: m/z = 511.3 [M-1-4HBr]$^-$; Anal. Calcd for $C_{22}H_{44}N_8O_4Br_4S$: C, 31.60; H, 5.30; N, 13.40. Found: C, 31.67; H, 5.24; N, 13.62.

8c: 0.318 g, 72%. Mp 235°–236 °C; $[\alpha]^{20}_D = +2.9$ ($c = 1.0$, H_2O); ^1H NMR (400 MHz, D_2O): δ 7.73–7.63 (m, 1 H, uracil-6-CH), 7.25–7.20 (m, 2 H, Ph-H), 6.96–6.87 (m, 2 H, Ph-H), 4.66–4.54 (m, 1 H, CHCON), 4.48–4.39 (m, 1 H, NH_2CH), 3.80–3.23 (m, 16 H, cyclen-CH_2),



Scheme 1 Synthetic route of uracil-PNA monomer-cyclen conjugates.

3.20–3.17 (m, 2 H, Ph-C$\underline{H}_2$), 3.14–3.07 (m, 2 H, S-C$\underline{H}_2$-uracil), 3.04–2.97 (m, 2 H, NCHC$\underline{H}_2$S); ESI-MS: m/z = 561.3 [M-1-4HBr]$^-$; Anal. Calcd for $C_{25}H_{42}N_8O_5Br_4S$: C, 33.88; H, 4.78; N, 12.64. Found: C, 33.69; H, 4.92; N, 12.70.

General Procedure for the Preparation of Zinc(II) Complex 9

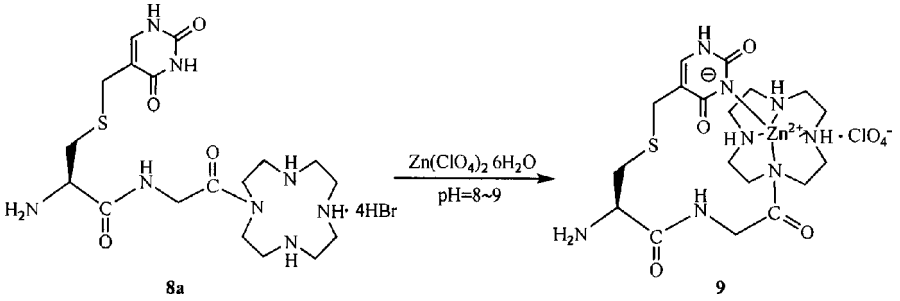
To an aqueous solution (5 ml) of compound **8a** (0.272 g, 0.350 mmol), an aqueous solution (5 ml) of $Zn(ClO_4)_2{\cdot}6H_2O$ (0.134 g, 0.360 mmol) was added slowly. The pH of the solution was adjusted to 8–9 with 1 M NaOH. After being stirred overnight, the reaction mixture was concentrated under reduced pressure. The obtained residue was crystallized from water to afford zinc(II) complex **9** as a white solid (0.180 g, 83%). Mp 270°–272 °C; $[\alpha]^{20}_D$ = +2.5 (c = 1.0, H_2O); ^{1}H NMR (400 MHz, DMSO-d_6): 10.23 (d, 1 H, J = 5.2 Hz, uracil-3-N$\underline{H}$), 7.50 (d, 1 H, J = 8.4 Hz, CON$\underline{H}$), 7.18 (d, 1 H, J = 5.6 Hz, uracil-6-C$\underline{H}$), 6.13 (s, 2 H, N$\underline{H}_2$), 4.41–4.38 (m, 1 H, NH_2C$\underline{H}$), 4.22–4.17 (m, 2 H, C$\underline{H}_2$CO), 3.67–3.62 (m, 3 H, CH_2N$\underline{H}$C$\underline{H}_2$), 3.51–3.20 (m, 16 H, cyclen-C$\underline{H}_2$), 2.95–2.80 (m,

2 H, S-C$\underline{H_2}$-uracil), 2.51–2.49 (m, 2 H, NCHC$\underline{H_2}$S); HRMS (ESI) calcd for $C_{18}H_{31}N_8O_4SZn$ $[M\text{-}ClO_4]^+$: $m/z = 519.1475$. Found: 519.1475; Anal. Calcd for $C_{18}H_{31}N_8O_8ClSZn$: C, 34.85; H, 5.04; N, 18.06. Found: C, 34.73; H, 5.06; N, 17.92.

RESULTS AND DISCUSSION

Known methods for the synthesis of PNA monomers rely on the use of protecting groups for the amino function and for the nucleobases [9,21]. By using 5-hydroxymethyluracil as a starting material, thio-PNA monomer was obtained through the reaction of the mercapto group on the side chain of L-cysteine with the hydroxylmethyl group at the 5-position of uracil, so in this study no protecting group was needed for the nucleobases. Treatment of L-cysteine hydrochloride **1** with 5-hydroxymethyluracil **2** in 2 N HCl produced the L-cysteine derivative **3** in 94% yield (Scheme 1). After the protection of the amino group of **3** with a Boc group, the dipeptides **5a–c** were afforded conveniently in high yield *via* the condensation of *N-tert*-butyloxycarbonyl-S-thyminylcysteine **4** with L-amino acid methyl ester hydrochloride in the presence of *N*-methyl morphine (NMM) and *i*-C_4H_9OCOCl. The compounds **5a–c** were then saponified with 2 N sodium hydroxide (and the reactions were monitored by TLC). The use of 5 equiv, of NaOH can make the saponification reaction speedly and complete. After the reaction mixtures were neutralized to pH = 7 with 1 N HCl, compounds **6a–c** were obtained in approximately quantitative yields and followed by condensation with 1,4,7-tris (*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecance (3Boc-cyclen) to give the cyclen-conjugated dipeptides **7a–c** in the presence of *N*-methyl morphine (NMM) and *i*-C_4H_9OCOCl in high yields. The Boc protective groups in **7a–c** were removed synchronously by 47.5% HBr in ethanol to give the desired product PNA monomer-cyclen conjugates **8a–c** in over 70% yield. It was also found that compounds **8a** (as free base) can rapidly bind Zn^{2+} in aqueous solution. As outlined in Scheme 2, compound **8a** was conveniently converted to the zinc(II) complex of uracil-PNA monomer-cyclen conjugate **9** in 83% yield. The structure of complex **9** was confirmed facilely by HRMS spectra (Figure 2), ^{1}H NMR spectra (Figure 3) and elemental analysis.

In summary, a synthetic route was developed to a novel uracil-PNA monomer appended with 1,4,7,10-tetraazacyclododecane, which will be used to produce PNA analogue oligomers containing special-site-substituted nucleobases as a nucleic acid mimic. Preliminary results demonstrated that the uracil-PNA monomer-cyclen conjugates can rapidly bind Zn^{2+} in aqueous solution, and the structure of the Zn(II) complex was confirmed facilely by HRMS spectra, ^{1}H NMR spectra and elemental analysis. The synthesis of the proposed oligomer and their hybridization properties will be reported in due course.



Scheme 2 Synthesis of zinc(II) complex of uracil-PNA monomer-cyclen conjugate **8a**.

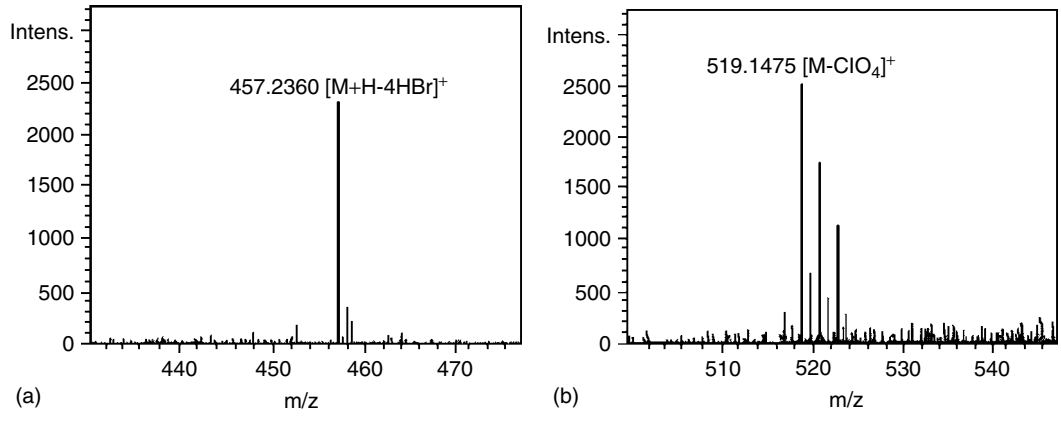


Figure 2 HRMS(ESI$^+$) spectrum of (a) **8a** (457.2340 in theory); (b) zinc(II) complex **9** (519.1475 in theory).

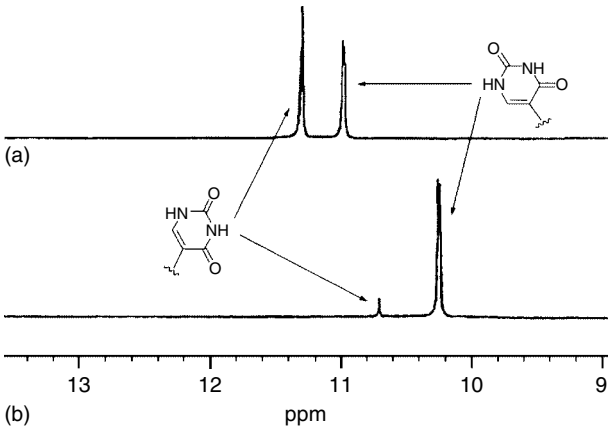


Figure 3 Spectral changes of amide-NH of uracil in ^{1}H NMR spectrum: (a) **8a**; (b) zinc(II) complex **9**.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (20132020, 20372051, 20471038), Program for New Century Excellent Talents in University, Specialized Research Fund for the Doctoral Program of Higher Education and Scientific Fund of Sichuan Province for Outstanding Young Scientist.

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